

Two New Species of *Diplosoma* (Ascidiacea: Didemnidae) Bearing Prokaryotic Algae *Prochloron* from Okinawajima (Ryukyu Archipelago, Japan)

Atsushi T. Oka, Mayu Suetsugu and Euichi Hirose*

Department of Chemistry, Biology and Marine Science, University of the Ryukyus,
Senbaru 1, Nishihara, Okinawa 903-0213, Japan

ABSTRACT—Two new species of didemnid ascidians, *Diplosoma ooru* sp. nov. and *Diplosoma simileguwa* sp. nov., are described from coral reefs on Okinawajima (Ryukyu Archipelago, Japan). These two species form green colonies, having a symbiotic association with a prokaryotic alga *Prochloron* sp. The former species was found at the reef edges in the subtidal zone and the latter was found in a shallow reef lagoon. In these species, the colonies are thinner and the zooids are smaller than those of any other *Prochloron*-bearing *Diplosoma* species so far described. Moreover, each of the present new species has a unique combination of stigmatic numbers: 5 stigmata in the first and third rows, 6 in the second row, and 4 in the fourth in *D. ooru*; 4 stigmata in the first and third rows, 5 in the second row, and 3 in the fourth in *D. simileguwa*. In both of the new species, the retractor muscle emerges from the underside of the thorax. Larval morphology of *D. ooru* is also described.

Key words: *Diplosoma*, Didemnidae, new species, algal symbiosis, Okinawajima

INTRODUCTION

According to Berrill (1950), the members of the family Didemnidae are colonial ascidians with small zooids divided into thorax and abdomen. There are 3 or 4 rows of stigmata in the thorax and calcareous spicules are generally present in the tunic. The most distinctive feature of the didemnids is their unique mode of asexual reproduction, i.e., pyloric budding. Didemnid ascidians are widely distributed from tropical to temperate waters, and several species have been reported from the Antarctic (Kott, 1969). In recent decades, many new didemnid species have been described, mostly in tropical waters (e.g., Monniot and Monniot, 1987, 2001; Kott, 2001, 2004a, 2004b).

Genera of the Didemnidae are generally distinguished by the forms of the testis and the vas deferens (the beginning part of the sperm duct), atrial aperture, and the number of rows of stigmata. To date, six didemnid genera have been reported from Japan (Nishikawa, 1995). Among them, the members of the genus *Diplosoma* are characterized by the 4 rows of stigmata, the uncoiled vas deferens, and the lack of calcareous spicules in the tunic (Cf. Berrill, 1950). Only two *Diplosoma* species have been previously reported from

Japan: *Diplosoma mitsukurii* Oka 1892 and *Diplosoma midori* (Tokiooka, 1954). The former is a common colonial ascidian distributed along the Japanese coast; there are taxonomic arguments that this species may be a junior synonym of a cosmopolitan congener, *D. listerianum* (Milne Edwards, 1841), although some larval features distinguish this species from other congeners (Nishikawa, 1990). The latter is a photosymbiotic species distributed in (sub)tropical water in Japan, but Kott (2001) claimed that *D. midori* is a junior synonym of *Diplosoma simile* (Sluiter, 1909).

In (sub-) tropical waters, some didemnid ascidians are associated with the phototrophic symbiont, *Prochloron*, a prokaryotic alga with the same chlorophyll pigments, chlorophyll *a* and *b*, as those found in the chloroplasts of green algae and higher plants (reviewed in Lewin and Cheng, 1989). The taxonomic studies of *Prochloron*-bearing didemnids have been carried out mainly around the Great Barrier Reef and tropical islands in the West Pacific (Eldredge, 1966; Kott, 1977, 1980, 1982, 2001; Monniot and Monniot, 1987, 1996, 2001), whereas there are few reports from Japan and its adjacent waters (Tokiooka, 1954; Nishikawa, 1995). The Ryukyu Archipelago is situated in the southern most part of Japan and near the north limit of coral reefs in the West Pacific. Recently we investigated the distribution of some *Prochloron*-bearing didemnids on Iriomotejima in the south Ryukyus (Hirose *et al.*, 2004), and found some of the

* Corresponding author. Phone: +81-98-895-8880;
Fax : +81-98-895-8576;
E-mail: euichi@sci.u-ryukyu.ac.jp

species including *Diplosoma* spp. new to Japan. This may indicate that more taxonomic and biogeographical studies of *Prochloron*-bearing ascidians in the Ryukyu Archipelago are still needed. We here describe two new species of *Diplosoma* associated with *Prochloron* from Okinawajima in the middle Ryukyus.

MATERIALS AND METHODS

The ascidian colonies were collected from the subtidal parts of coral reefs in 2003 and 2004 in Bise, Ginan-zaki, Kayo, Odo and Teniya (Okinawajima, Ryukyu Archipelago, Japan) by snorkeling (< ca. 1 m deep at the lowest tide) (Fig. 1). Some colonies of *Diplosoma simile* and *D. virens* were collected in the same sites for comparisons of the zooid morphology. The colonies, scraped off the substratum with a steel knife, were put into a 300-ml plastic bottle with seawater and immediately brought to the laboratory. The specimens were anesthetized with menthol and 0.37 M magnesium chloride, and then fixed in 10% formalin-seawater. Zooids and embryos were isolated from the fixed colonies by dissection under a stereomicroscope. More than 4 zooids were examined for each colony. Some fixed specimens were stained with rose bengal. All the drawings were prepared under a stereomicroscope or light microscope equipped with a camera lucida. Some of the examined specimens were preserved in 70% ethanol and deposited as type specimens in the National Science Museum Tokyo (NSMT) and the Nagoya University Museum (NUM).

For histological investigation, some fixed colonies were cut into pieces and postfixed with 1% osmium tetroxide. They were dehydrated through an ethanol series, cleared with *n*-butyl glycidyl ether, and embedded in low-viscosity epoxy resin. Sections 0.8–1.5 μ m thick were stained with toluidine blue.

DESCRIPTION

Family Didemnidae Giard, 1872

Genus *Diplosoma* MacDonald, 1859

The genus *Diplosoma* is characterized by the lack of

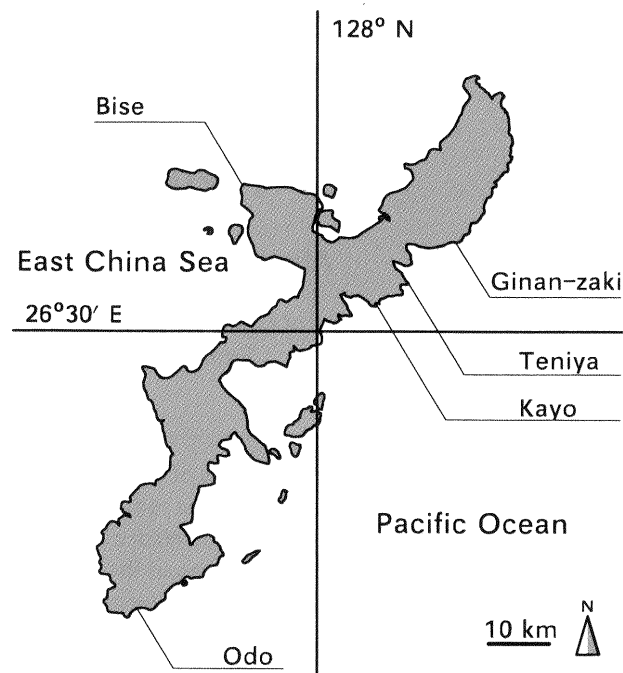


Fig. 1. Map of Okinawajima showing sampling localities.

spicules, absence of atrial siphon, 4 rows of stigmata, the uncoiled vas deferens, and undivided or two-lobed testis. The two new species described below possess these features and readily fall into this genus.

Diplosoma ooruu Hirose et Suetsugu sp. nov.
(Fig. 2A and 3)

Specimens

Holotype: NSMT Pc-1105, colony from the reef edge at Teniya collected on 13 June 2003. An irregularly shaped

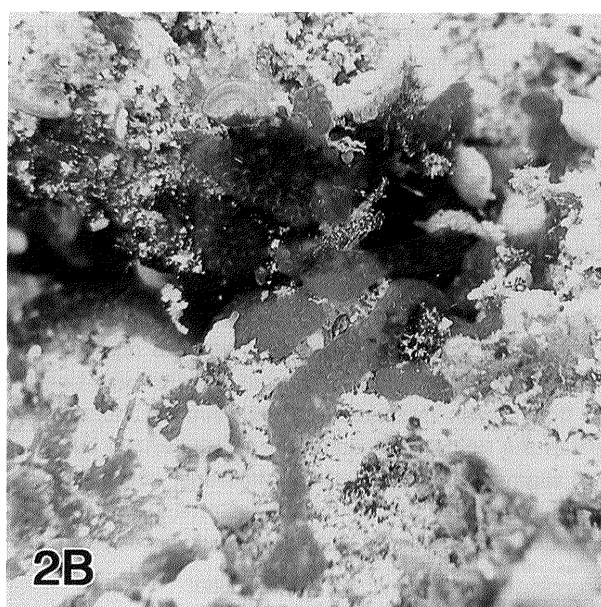
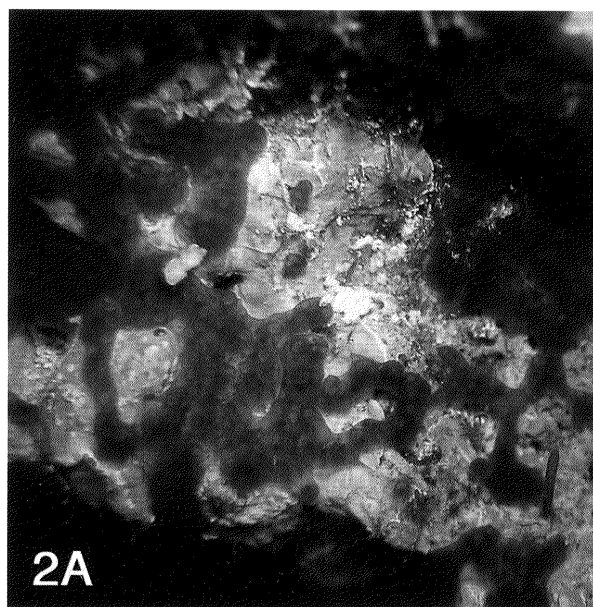


Fig. 2. Colonies of *Diplosoma ooruu* sp. nov. on the reef edge at Bise (A) and *Diplosoma simileguwa* sp. nov. in the reef lagoon at Teniya (B).

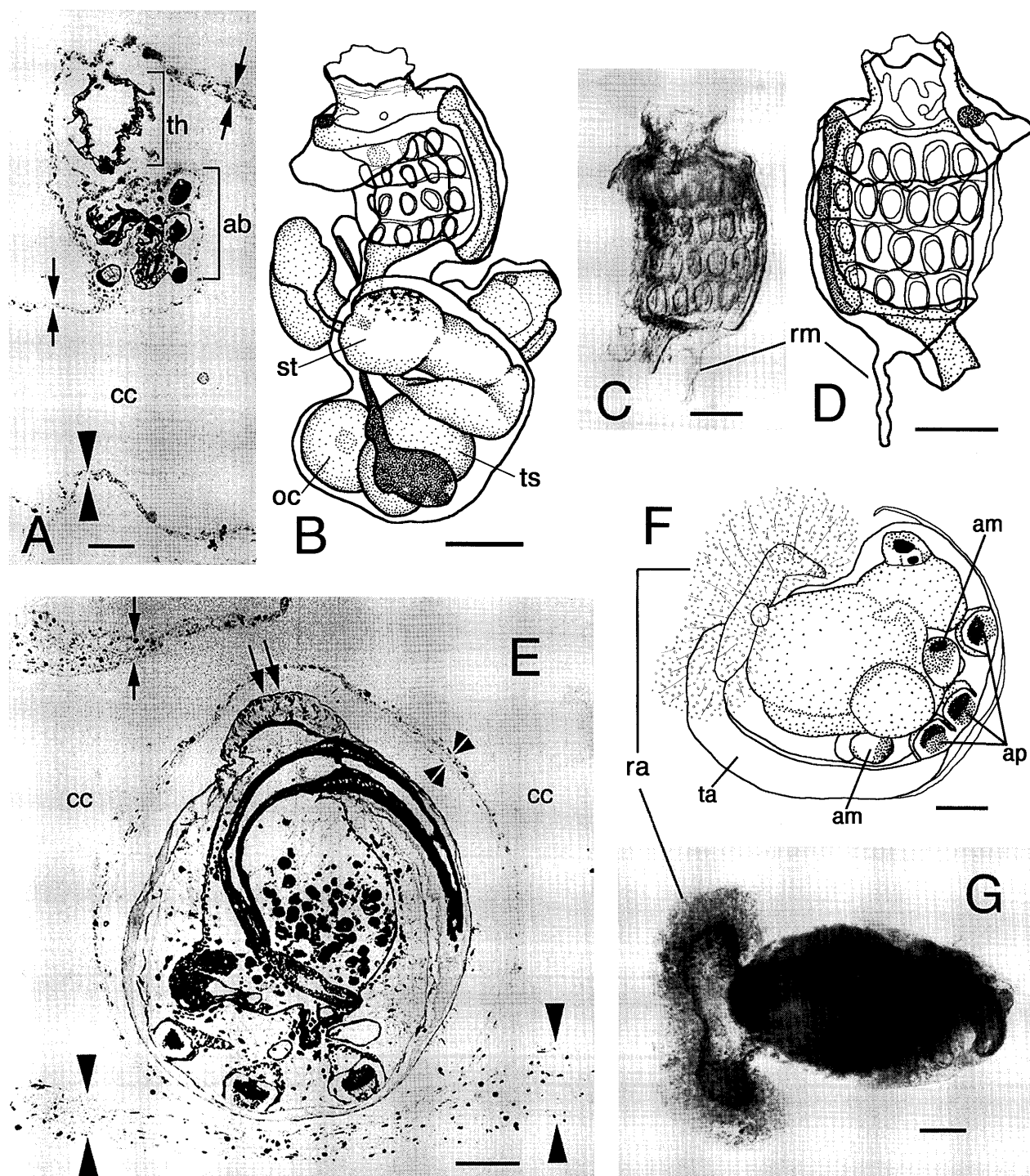


Fig. 3. *Diplosoma ooru* sp. nov.: **A**, Cross section of a colony; **B**, Zooid (right side) from the holotype colony (NSMT Pc-1105); **C**, Thorax (right side); **D**, Thorax (left side); **E**, Cross section of an embryo brooded in a colony; **F**, Pre-hatching larva (right side) from a paratype colony (NSMT Pc-1106); **G**, Pre-hatching larva (ventral side). Arrows, upper tunic layer; arrowheads, basal tunic layer; double arrows, primordial rastrum; double arrowheads, tunic layer surrounding the embryo; ab, abdomen; ap, adhesive papillae; am, ampullae; cc, cloacal cavity; oc, oocyte; ra, rastrum; rm, retractor muscle; st, stomach; ta, tail; th, thorax; ts, testis. All scale bars indicate 0.1 mm.

colony fragment about 15 mm long containing ca. 100 zooids and 9 tailed embryos.

Paratypes: NUM-Az 0394, colony from the reef edge at Bise collected on 14 June 2003; NSMT Pc-1106, colony from the reef slope at Ginan-zaki on 29 July 2003; NUM-Az

0395, colony from the reef edge at Teniya on 28 August 2003.

Collection sites

Bise (May, June, July, August), Ginan-zaki (July), Kayo

(May), Odo (April, July) and Teniya (May, June, July, August) on Okinawajima.

Etymology

The specific epithet, “*ooru*”, refers to an Okinawan dialect word meaning green or blue, because the colony looks green due to the symbiotic algae.

Habitat

Colonies of *D. ooru* sp. nov. are common at the subtidal part of the reef edges (< ca. 1 m deep) on Okinawajima. Colonies attach to depressions of coral limestone at the reef edges of the subtidal zone and are often found with nongeniculate coralline algae. *Diplosoma simile* (Sluiter, 1905) and *Diplosoma virens* (Hartmeyer, 1909) are found at the same site.

Colony

Along a groove on the substratum, the colony often forms a narrow sheet up to 40 mm in length. The thickness of the colonies is 0.5–0.8 mm. The colony is semitransparent green due to the symbiotic *Prochloron* cells in the cloacal cavity, so that the substratum surface is still partly visible. Colonies are attached to the substratum with thin cords of tunic extending from the colony base. There are one or two common cloacal openings. The ampullae of stolonial vessels are lined along the colonial margin. The ampullae are about 70 µm in diameter and 0.1–0.3 mm in length.

Zoooid

Zooids are sparsely distributed in the colony and easily taken out from a fixed colony. They are about 0.5 mm long (0.3 mm thorax and 0.2 mm abdomen) (Fig. 3A). In preservative, zooids are whitish, while stigmatic rims, intestine, and oocytes are yellowish and testes are gray. The thorax often shrinks slightly in preservative (Fig. 3B). Each branchial siphon is six-lobed and the sphincter is distinct. Atrial aperture is wide. There are four rows of stigmata. In all the specimens examined (32 colonies), there are five stigmata in the first and third rows, six in the second row, and four in the fourth (Fig. 3C, D). The retractor muscle is short and emerges from the underside of the thorax. Buds emerge from the long esophagus. The abdomen curves near the bottom of the esophagus. The stomach is large and round, and often has brown scales on the surface. The intestine is long and curves at the rectum, which often contains several feces. Testes were found in some colonies collected between April and August. The testis is two-lobed, and the vas deferens runs straight along the border of the lobes. Oocytes were found in some colonies collected in June, July, and August.

Embryo and larva

Embryos are found in some colonies collected in June, July, and August. They could be clearly seen from the base of the colony, because the colonies are very thin. Mature

colonies usually possess several embryos including a few pre-hatching larvae. The pre-hatching larvae are more often found in July. Until the larvae spawn, each embryo develops in a pouch that consists of a thin layer of tunic connecting to the basal tunic of the parent colony (Fig. 3E). The pouch insulates the embryo from the common cloacal cavity where *Prochloron* cells are distributed. In the early tailed embryos, the primordial rastrum is directed upward (double arrows in Fig. 3E). The embryo attains 0.5 mm in diameter, with the tail wound about halfway around the trunk. There are three adhesive papillae with short stalks and two pairs of ampullae. In pre-hatching larvae, a well developed rastrum projects from the postero-dorsal end of the larval trunk, just above the basal point of the tail (Fig. 3F, G). Numerous *Prochloron* cells are attached to the hairy surface of the rastrum.

Remarks

Diplosoma ooru sp. nov. is characterized by the very small zooids (about 0.5 mm long), and the unique combination of stigmatic numbers (Fig. 3B, D). Whereas didemnid ascidians in general have small zooids as compared to other colonial ascidians, *Diplosoma* zooids 0.5 mm long are exceptional. Moreover, the stigmatic number per row is usually 5 or more in both symbiotic and non-symbiotic *Diplosoma* spp. so far described on their stigmatic numbers. It is unlikely that the immaturity of the specimens was responsible for their small zooids and small stigmatic numbers, because some specimens were sexually mature and contained pre-hatching larvae.

In the genus *Diplosoma*, 6 species have been described as *Prochloron*-bearings. They are *D. simile* (Sluiter, 1909), *D. virens* (Hartmeyer, 1909), *D. midori* (Tokiooka, 1954), *D. multipapillatum* Kott, 1980, *D. matie* Monniot and Monniot, 1987, and *D. pavonia* Monniot and Monniot, 1987 (Table 1). Kott (2001), however, considered that *D. midori* is a junior synonym of *D. simile* and both *D. matie* and *D. pavonia* are junior synonyms of *D. virens*. Among the 6 species, the present species shares the following features with *D. simile*: *Prochloron* cells distributed in common cloacal cavity, retractor muscle extending from underside of thorax, and three adhesive papillae present in larva. *Diplosoma simile* is readily distinguished from the present new species by the following character states; colonies are 1.0–2.5 mm thick, zooids are about 1 mm long, and the branchial sac has six stigmata in each row or five stigmata in the fourth row and six in the other rows. We collected *D. simile* colonies from the same sites where the new species was found, and we verified that the zooids always have more than 4 stigmata per row even in small colonies containing less than 10 zooids. According to the original description, the colony of *Diplosoma midori* is thicker (1.0–3.0 mm) than that of the new species and the branchial sac has five stigmata in the fourth row and six in the other rows (Tokiooka, 1954).

Due to the small zooid and thin tunic layer, this species has very thin colonies (0.5–0.8 mm thick), and the embryos

Table 1. Features of the new species and other *Prochloron*-bearing *Diplosoma* species

Species	Colony thickness	Zooid length ¹	Stigmata ²	Retractor muscle ³	Larval trunk	Adhesive papillae	Larval ampullae	References
<i>D. ooru</i>	0.5–0.8 mm	ca. 0.5 mm	5654	T	0.5 mm	3	2 pairs	present study
<i>D. simileguwa</i>	1.0–1.5 mm	0.5–0.6 mm	4543	T	–	–	–	present study
<i>D. midori</i>	1–3 mm	0.9 mm*	6665	?	–	–	–	Tokioka (1954)
<i>D. simile</i>	>1 mm	0.8 mm	6665	–	–	–	–	Sluiter (1909)
<i>D. simile</i>	<2 mm	1 mm	6666	T	0.8 mm	3	3 pairs	Kott (1980)
<i>D. simile</i>	–	0.9 mm*	6666/6665	T	–	3*	3 pairs*	Monniot and Monniot (1987)
<i>D. simile</i>	ca. 2 mm	ca. 1.0 mm	6666	T	0.8 mm	3	3–4 pairs	Kott (2001)
<i>D. virens</i>	ca. 1 cm	0.5 mm	5665/6666*	–	–	–	–	Herdman (1906)
<i>D. virens</i>	1.5–2 mm	<1.5 mm	5/6**	–	–	–	–	Tokioka (1942)
<i>D. virens</i>	<4 mm	<1.1 mm	6666	E?	772 µm	3	2 pairs	Eldredge (1966)
<i>D. virens</i>	2–8 mm	ca. 1 mm	6666	E	1.5 mm	3	2–3 pairs	Kott (1982)
<i>D. virens</i>	2–5 mm	ca. 1 mm	5/6**	E	1.5 mm	3–8	2–4 pairs	Kott (2001)
<i>D. multipapillatum</i>	<1.5 mm	ca. 0.8 mm	5555	E	ca. 1.3 mm	30	3 pairs	Kott (1980)
<i>D. matie</i>	3 mm	ca. 1 mm*	6665	E	<850 µm	5–12	5–6 pairs	Monniot and Monniot (1987)
<i>D. pavonia</i>	<5 mm	0.9 mm*	6666	E	1.15 mm	5–8	2 pairs	Monniot and Monniot (1987)

¹, Gonads are not included.², Numbers of stigmata from the first row to the fourth row.³, Retractor muscle extending from the the zooid at the underside of the thorax (T) or halfway along the esophagus (E).

*, Measured from the figures in the references.

**, Stigmata 5–6 per row.

are small and develop in a pouch that bulges from the thin basal tunic into the cloacal cavity (Fig. 3E), whereas in *D. simile* (Hirose, 2000) and *D. virens* (Hirose *et al.*, in press), sympatric congeners of the new species, the embryos are totally embedded in the basal tunic layer.

Diplosoma simileguwa Oka et Hirose sp. nov.
(Fig. 2B, Fig. 4)

Specimens

Holotype: NSMT Pc-1107, colony from the reef lagoon at Teniya collected on 13 July 2003. A colony fragment (ca. 5 x 2 mm) containing 30 zooids.

Paratypes: NUM-Az 0396, colony from the reef lagoon at Teniya collected on 13 July 2003; NUM-Az 0397, colony from the reef lagoon at Teniya on 7 May 2004.

Collection sites

Ginan-zaki (July) and Teniya (May, June, July) on Okinawajima.

Etymology

The present species is similar in morphology to *Diplosoma simile* but has smaller zooids and fewer branchial stigmata. The suffix of the specific epithet, “-guwa”, is a suffix in Okinawan dialects meaning small.

Habitat

The present new species were found on the reef slope at Ginan-zaki and in the reef lagoon at Teniya, Okinawajima. Colonies are not common and are inconspicuous because

of their small size. They attach on coral limestone or coral-ines in a shallow reef lagoon (< 0.5 m deep at the lowest tide). *Diplosoma simile* and *D. virens* are often found at the same sites.

Colony

The colony forms a small sphere up to 4 mm in diameter or an irregular sheet less than 10 mm long. The thickness of colonies is about 1.0–1.5 mm. The colony is semi-transparent green due to the symbiotic *Prochloron* cells distributed in the cloacal cavity. The rims of some branchial apertures are light blue. There is a common cloacal opening at the center of the upper surface of the colony. Colonies are attached to the substratum with thin cords of tunic extending from the base. The ampullae of stolonial vessels, lined along the colonial margin, are about 70 µm in diameter and 0.1–0.3 mm in length.

Zooid

Zooids are sparsely distributed in the colony and easily taken out from a fixed colony. They are 0.5–0.6 mm long (0.3–0.4 mm thorax and ca. 0.2 mm abdomen) (Fig. 4A, B). In preservative, zooids are whitish, while stigmatic rims, intestine, and oocytes are yellowish and testes are gray. The branchial siphon is six-lobed and the sphincter is distinct. The atrial aperture is wide. There are four rows of stigmata. In all the 8 specimens so far examined, there are four stigmata in the first and third rows, five in the second row, and three in the fourth (Fig. 4C, D). The retractor muscle is short and emerges from the underside of the thorax. Buds

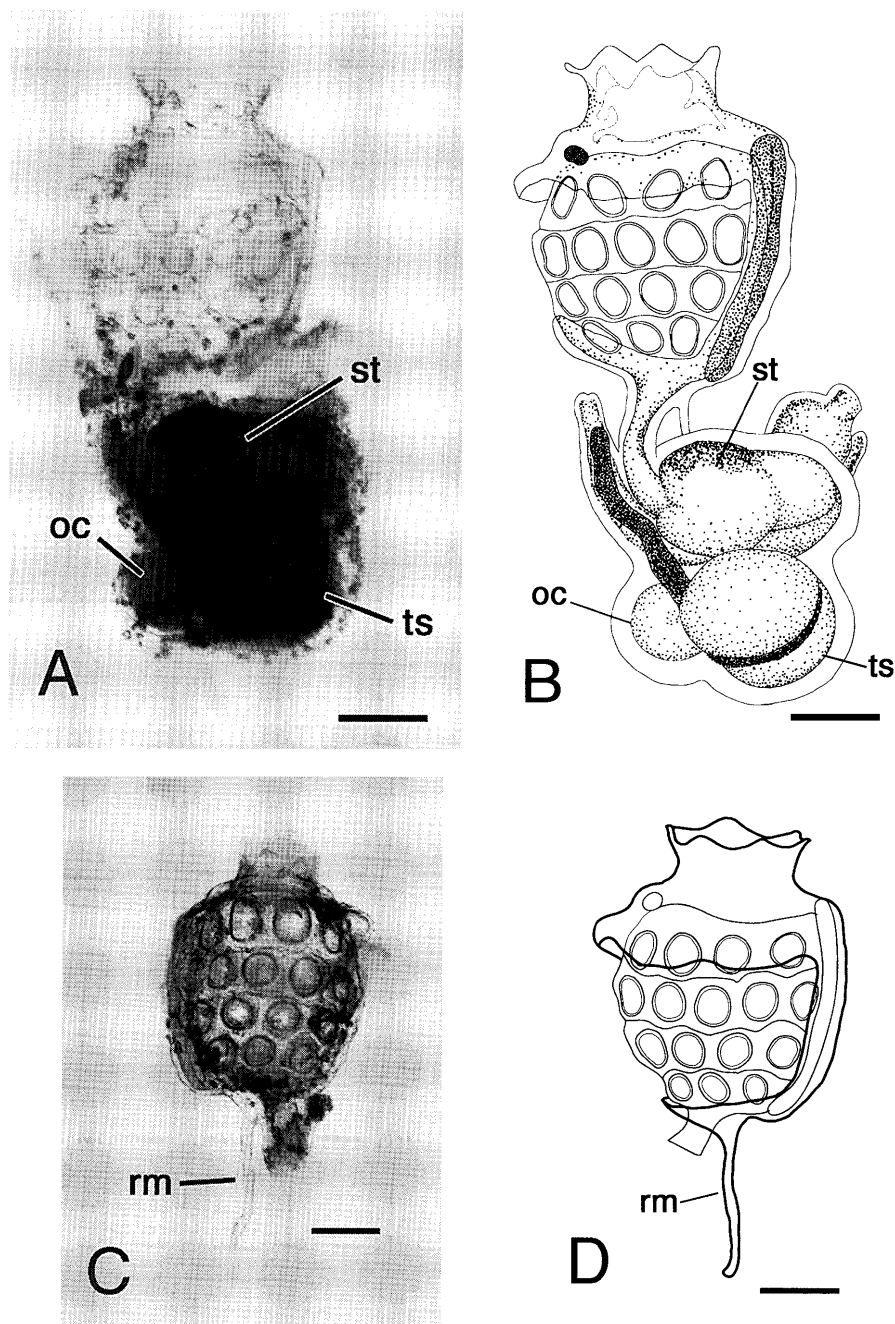


Fig. 4. *Diplosoma simileguwa* sp. nov. Photographs and drawings of zooid and thorax from holotype colony (NSMT Pc-1107): **A**, Zooid (right side); **B**, Zooid (right side); **C**, Thorax (left side); **D**, Thorax (right side). oc, oocyte; rm, retractor muscle; st, stomach; ts, testis. All scale bars indicate 0.1 mm.

emerge from the long esophagus. The abdomen curves near the bottom of the esophagus. The large stomach is round. Dark brown scales, often attached to the upper surface of the stomach, form a brownish patch on the stomach of each zooid, which is prominent in formalin-fixed specimens. The intestine is long and curves at the rectum, which often contains several feces. Testes were found in the specimens collected in July. The testis is two-lobed and the vas deferens runs straight along the border of the lobes. Oocytes were found in the specimens collected in July.

Embryos or larvae have not been found in the colonies so far examined.

Remarks

Diplosoma simileguwa sp. nov. is characterized by the very small zooids and the unique combination of stigmatic numbers. In particular, the 4th row with only 3 stigmata is a unique feature of this *Diplosoma* species. Since some of the specimens were sexually mature and the zooids possessed testes and oocytes, the small zooids and lower numbers of

stigmata per row could probably not be attributed to the immaturity of the colonies examined.

Among the *Prochloron*-bearing *Diplosoma* spp., the present species shares the following features with *D. ooru* sp. nov.: small zooids (0.5–0.6 mm long), and the retractor muscle emerging from under the thorax. In *D. simileguwa* sp. nov., the combination of the stigmatic numbers in the branchial basket is different from those in *D. ooru* sp. nov. and other *Diplosoma* species. The colony of *D. simileguwa* is usually a small sphere or an irregular sheet, while that of *D. ooru* is rather a narrow sheet. In *D. simileguwa*, the common cloacal cavities are usually larger and thus the colonies are thicker than those of *D. ooru*.

DISCUSSION

As compared to the other *Prochloron*-bearing *Diplosoma* spp., *D. ooru* sp. nov. and *D. simileguwa* sp. nov. have relatively small zooids (about 0.5 mm long) and the unique combinations of the stigmatic numbers that are constant within each species (Table 1). Although there may be some intraspecific variation in the stigmatic numbers per row in some *Diplosoma* spp., no *Prochloron*-bearing *Diplosoma* other than the present species have been reported to have less than 5 stigmata per row.

In *D. simile* and the present two species, the retractor muscle extends from the underside of the thorax, whereas in the other *Prochloron*-bearing *Diplosoma* spp. except possibly for *D. midori* it extends from the zooid halfway along the esophagus. This may indicate that *D. simile* and the present species are phylogenetically more closely related than the other *Diplosoma* species. The stigmatic numbers clearly indicate a distinction between *D. midori* and both of the present species, although the systematic position of *D. midori* is still open to question. It is difficult to discriminate between *D. midori* and *D. simile* due to the lack of descriptions of larvae and retractor muscles in *D. midori*, and Kott (2001) assigned *D. midori* as a junior synonym of *D. simile*. We examined the syntype specimens of *D. midori*, but could not conclude either way, because the zooids were too poorly preserved.

Symbiotic relationships between didemnids and *Prochloron* have so far been reported in four genera (*Didemnum*, *Diplosoma*, *Lissoclinum* and *Trididemnum*), although each genus also includes non-symbiotic species. Therefore, the symbiosis might have had an independent origin at least once in each genus (Kott, 1980, 1982). While the larvae of the host didemnids usually bear *Prochloron* cells and thereby transmit the symbiont to the next generation (vertical transmission), the larval adaptations for algal transmission are different among the ascidian genera (Kott, 1977, 1980, 1981, 1982). In the *Prochloron*-bearing *Diplosoma* species, Kott (1980, 1981, 1982) described an organ called the rastrum (plant rake), a two-lobed projection from the postero-dorsal part of the larval trunk that bears *Prochloron* cells from the mother colony in *D. multipapillatum*, *D.*

simile, and *D. virens*. Thereafter, the rastrum was found in the larvae of *D. matie* and *D. pavonia* (Monniot and Monniot, 1987, 2001). The larvae of *D. ooru* also possess a rastrum, whereas a rastrum has never been described in any *Prochloron*-bearing species of the other genera. Therefore, the rastrum is apparently a synapomorphic character of the *Prochloron*-bearing *Diplosoma* species.

Both of the new species were collected from coral reefs on Okinawajima in the middle Ryukyus. In particular, *D. ooru* is common at the reef edges of this area. Evidently our knowledge of didemnid taxonomy and distribution in subtropical East Asian islands is still poor.

ACKNOWLEDGMENTS

We are indebted to Dr. Ralph A. Lewin and Dr. Tohru Iseto for their valuable comments. Our thanks are also due to Professor Hidetoshi Ota for his advice on nomenclature and Mr. Takumi Fukuda for providing some specimens. The present study was partly supported by Japan-Australia Research Cooperative Program, Grant-in-Aid for Scientific Research (#16570081) from Japan Society for the Promotion of Science, and the 21st Century COE program of the University of the Ryukyus.

REFERENCES

- Berrill NJ (1950) The Tunicata with an account of the British species. Ray Society, London
- Eldredge LG (1966) A taxonomic review of Indo-Pacific didemnid ascidians and descriptions of 23 central Pacific species. *Micronesica* 2: 161–261
- Hartmeyer R (1909) Ascidiaceen. In "Klassen und Ordnungen des Tierreichs, vol 3 suppl. part 89–98" Ed by HG Bornn, CF Winter, Leipzig, pp 1281–1772
- Herdman WA (1906) Report on the Tunicata. Report to the Government of Ceylon on the Pearl Oyster Fisheries of the Gulf of Manaar Supplementary Reports 39: 295–348
- Hirose E (2000) Plant rake and algal pouch of the larvae in the tropical ascidian *Diplosoma similis*: an adaptation for vertical transmission of photosynthetic symbionts *Prochloron*. *Zool Sci* 17: 233–240
- Hirose E, Akahori M, Oka AT, Kurabayashi A (2004) Some *Prochloron*-bearing didemnid ascidians collected from the reef shores of Iriomote Island (Okinawa, Japan). *Biol Mag Okinawa* 42: 7–15
- Hirose E, Oka AT, Akahori M (2005) Sexual reproduction of the photosymbiotic ascidian *Diplosoma virens* in the Ryukyu Archipelago, Japan: vertical transmission, seasonal change, and possible impact of parasitic copepods. *Mar Biol* (in press) (DOI:10.1007/s00227-004-1469-5)
- Kott P (1969) Antarctic Ascidiacea. *Antarct Res Ser Washington* 13: 1–239
- Kott P (1977) Algal-supporting didemnid ascidians of the Great Barrier Reef. *Proc Third Internat Coral Reef Symp* 1: 615–621
- Kott P (1980) Algal-bearing didemnid ascidians in the Indo-West Pacific. *Mem Qld Mus* 20: 22–33
- Kott P (1981) Didemnid-algal symbiosis: Algal transfer to a new host generation. *Proc Fourth Internat Coral Reef Symp* 2: 721–723
- Kott P (1982) Didemnid-algal symbiosis: Host species in the Western Pacific with notes on the symbiosis. *Micronesica* 18: 95–127
- Kott P (2001) The Australian Ascidiacea, part 4, Aplousobranchia (3), Didemnidae. *Mem Qld Mus* 47: 1–408

- Kott P (2004a) New and little-known species of Didemnidae (Ascidacea, Tunicata) from Australia (part 1). *J Natur History* 38: 731–774
- Kott P (2004b) New and little-known species of Didemnidae (Ascidacea, Tunicata) from Australia (part 2). *J Natur History* 38: 2455–2526
- Lewin RA, Cheng L (1989) *Prochloron*. A Microbial Enigma. Chapman and Hall, New York
- Milne Edwards H (1841) Observations sur les ascidies composées des côtes de la Manche. *Mem Acad Sci Paris* 18: 217–326
- Monniot C, Monniot F (1987) Les ascidies de Polynésie française. *Mém Mus Natn Hist Nat A* 136: 1–155
- Monniot F, Monniot C (1996) New collection of ascidians from the Western Pacific and Southeast Asia. *Micronesica* 29: 133–279
- Monniot F, Monniot C (2001) Ascidians from the tropical western Pacific. *Zoosystema* 23: 201–383
- Nishikawa T (1990) The ascidians of the Japan Sea. I. *Publ Seto Mar Biol Lab* 34: 73–148
- Nishikawa T (1995) Phylum Chordata. In “Guide to seashore animals of Japan with color plates and keys. II” Ed by S Nishimura, Hoiku-sha, Osaka, pp 573–610 (In Japanese)
- Oka A (1892) Die periodische Regeneration der oberen Körperhäfte bei den Diplosomiden. *Biol Centr* 12: 265–268
- Sluiter CP (1909) Die Tunicaten der *Siboga*-Expedition Part II, Die merosomen Ascidien. *Siboga Expeditie* 56B: 1–112
- Tokioka T (1942) Ascidians found on the mangrove trees in Iwayama Bay, Palao. *Palao Trop Biol Stn Stud* 2: 497–507
- Tokioka T (1954) Invertebrate fauna of the intertidal zone of the Tokara Islands. VII. Ascidians. *Publ Seto Mar Biol Lab* 3: 239–264

(Received July 2, 2004 / Accepted December 24, 2004)